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Chimeric Primates: Embryonic Stem Cells Need Not Apply

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In this issue, Tachibana et al. report the generation of the first chimeras from a nonhuman primate, the rhesus monkey. Unlike mice, rhesus chimeras fail to form when embryonic stem cells are injected into blastocysts. Instead, chimera formation is achieved by aggregation of several four-cell embryos.

Pluripotency, or the ability of an unspecialized cell to give rise to all cell types of the body, is a property of early embryonic cells before they progress toward increased lineage restriction. The inner cell mass (ICM) of the blastocyst and the epiblast of the postimplantation embryo are comprised of pluripotent cells, both in vivo and when isolated and cultured in vitro. Various assays prove that human embryonic stem cells (hESCs), the in vitro isolates of the ICM, are pluripotent, as they can give rise to representatives of all three germ layers in vitro and in teratomas. However, a more stringent assay routinely used in mice, the production of chimeras by blastocyst injection to confirm that injected ESCs functionally contribute to all cell types of the body, cannot ethically be done in the human. Mitalipov and colleagues (Tachibana et al., 2012) have now used the rhesus monkey to examine the question of whether primate ESCs can contribute to chimeras.

Tachibana et al. (2012) report that, unlike in the mouse system, monkey ESCs are unable to contribute to chimerism when injected into monkey host blastocysts or into four-cell embryos (Figure 1). To rule out technical problems, the authors transfer whole ICM colonies into monkey blastocysts. Again, no chimeras formed, but remarkably, the injected ICM produced entire offspring, either as singletons or as one of monochorionic nonidentical twins. The ability to form offspring in this way has no precedence in mice. Although this finding is consistent with pluripotency of monkey ICM, it is also possible that the injected ICM already consists of lineage-restricted cell types and as a whole is capable of implanting using extraembryonic support from the host. Furthermore, this does not provide evidence that the ICM of the host blastocyst has the ability to functionally integrate injected cells to form a chimera. Instead, it took the aggregation of multiple very early stage four-cell embryos to show that embryonic chimerism in monkeys is possible at all.

As with all negative results, the inability of monkey ESCs to contribute to chimeras via blastocyst injection leaves unanswered whether their developmental potential, as this result suggests, is indeed more limited than that of mouse (m)ESCs, or if, with the right tweaking, a positive result may emerge in the future. In other words, could there be a barrier to chimerism that is unrelated to the potentiality of the injected cells? Perhaps the host environment is not conducive to chimera formation. Tachibana et al. provide evidence that at the time of ESC injections, the ICM in early monkey blastocysts differs from that of the early mouse blastocyst in that it has already segregated a primitive endoderm layer (future extraembryonic tissues) from an underlying epiblast (future embryo proper), a developmental event that does not occur until the late blastocyst stage in mice (Cockburn and Rossant, 2010). The relative positions of these two lineages are likely maintained by differential cell adhesion properties of the two cell populations and may be critical for organized embryo development. Consequently, cell mixing, a prerequisite for chimera formation, may not be tolerated. Interestingly, there is evidence that even in the mouse, ESCs do not efficiently contribute to late blastocysts (Ohta et al., 2008), possibly because the late blastocyst is no longer amenable to incorporating additional cells. The remarkable ability of whole ICM isolates when transplanted to continue development independently within the host blastocyst is further consistent with the notion that spatial information may already exist and is retained irrespective of the presence of other ICM cells.

Specific cell adhesion properties of the injected cells may also pose a barrier to cell mixing with host cells. Whereas mESCs tolerate single cell dissociation, hESCs do not, and recent evidence suggests that this is due to differences in signaling by E-cadherin, a cell adhesion molecule (Xu et al., 2010). Interestingly, Tachibana et al. report that dissociation of monkey ICM, like dissociation of hESCs, results in cell death. This apparent dependence of primate pluripotent cells on specific cell adhesion signaling may underlie at least in part their inability to mix with other cells and incorporate into hosts. It would be interesting to test whether small molecules that rescue single cell-dissociated hESCs (Xu et al., 2010) can overcome a potential barrier to cell mixing and enable chimera formation of dissociated monkey ESCs or ICM cells with monkey ICM.

The observation of relatively early lineage segregation into a primitive endoderm layer and an epiblast in monkey ICM also has implications for interpreting the developmental potential of derived ESCs. In fact, it is thought that primate

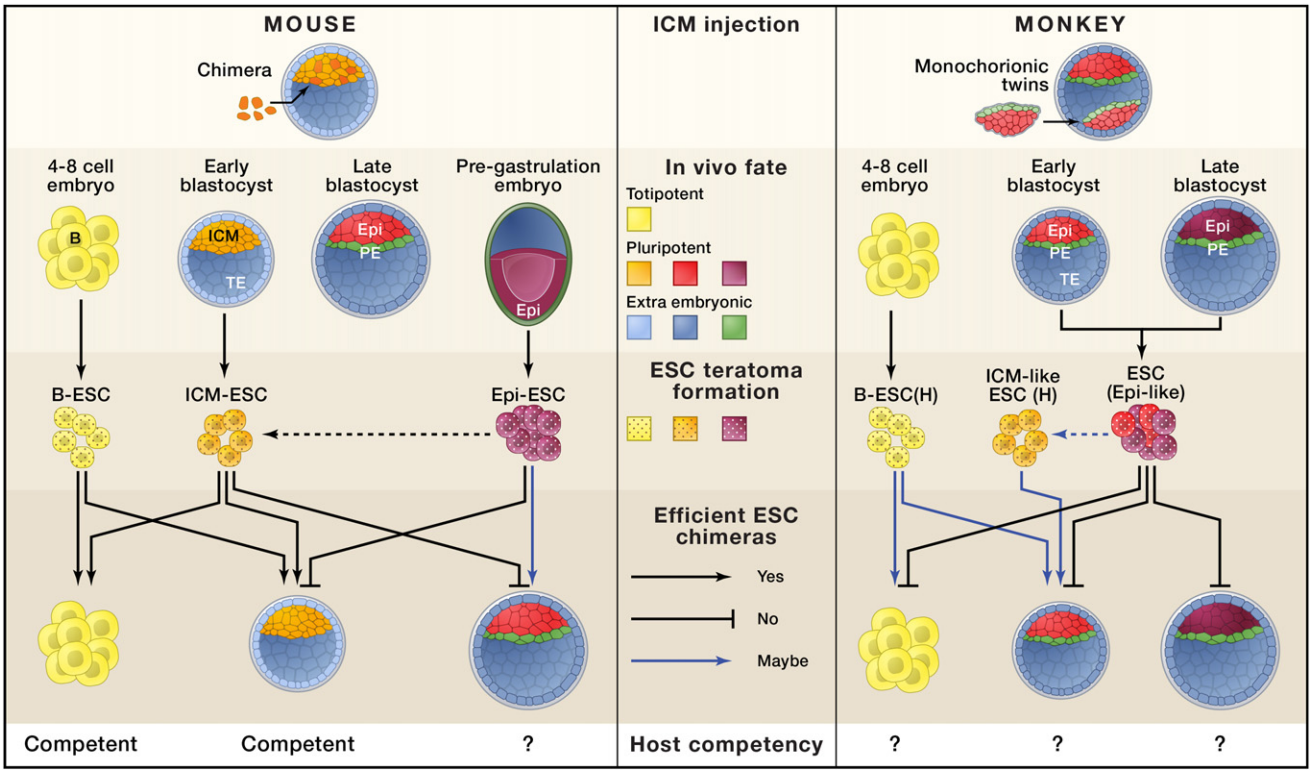

Figure 1. Mouse and Monkey ESCs Differ in Chimera-Forming Potential

In the mouse (left), pluripotent stem cell lines have been generated from blastomeres (B-ESC), the ICM of blastocysts (ICM-ESC), and the epiblast of post-implantation pregastrulation embryos (EpiSC). All form teratomas (Chung et al., 2006; Cockburn and Rossant, 2010; Nichols and Smith, 2011). B-ESCs and ICM-ESCs can contribute to chimeras when aggregated with eight-cell embryos or when injected into blastocysts. EpiSCs perform very poorly in the chimera assay when injected into early blastocysts. Given that early blastocysts are known to be chimera competent, this suggests that EpiSCs are not fully pluripotent, but it is also possible that host and injected cells are not developmentally compatible. It would be interesting to test whether EpiSCs can contribute to a late blastocyst as the nascent epiblast layer of that stage may be more compatible with EpiSC. Under appropriate culture conditions, EpiSCs can be converted to chimera-competent ICM-ESCs (Nichols and Smith, 2011). Dissociated ICM cells are capable of contributing to chimeras when injected into early blastocysts (Gardner, 1968).

In the monkey (right), cultured pluripotent stem cells have so far failed to contribute to chimeras. This is likely due to the developmentally advanced pluripotency of the ESCs (Epi-like). Converting existing ESCs to a more naive ICM-like state or generating B-ESCs may create chimera-competent ESCs, although it remains unclear whether the developmentally advanced early monkey blastocyst is chimera competent. Injected intact whole ICMs (dissociation causes cell death) fail to mix with host ICM but can form independent offspring (top).

B, blastomere; Epi, epiblast (embryonic ectoderm); ICM, inner cell mass; PE, primitive endoderm (hypoblast); TE, trophectoderm; (H) although not done in monkey, achieved in human; color shading indicates developmental fate/potency as indicated; darker shades represent more advanced developmental stages. Stippling indicates culture adaptation with largely unknown effects on developmental potency. Dashed arrow indicates phenotypic conversion in culture.

(monkey and human) ESCs are more equivalent to mouse epiblast stem cells, which are derived from postimplantation embryos and are developmentally advanced relative to naive mESCs (ICM-ESCs) (Nichols and Smith, 2011). This is an important consideration because mouse epiblast stem cells, although they can give rise to teratomas and are thus pluripotent, have only a very limited ability to contribute to chimeras. It is thus plausible that the pluripotency status, or chimera-forming ability, of ESCs may indeed be a reflection of the developmental status of the ICM—immature in mouse, more advanced in monkey—at the time of their derivation. However, an analysis of early embryo development in humans suggests that segregation of primitive endoderm and epiblast does not occur until the late blastocyst stage (Roode et al., 2011), whereas hESCs derived from an apparently more immature ICM still behave like developmentally advanced epiblast stem cells.

Interestingly, hESCs have been derived from blastomeres of early cleavage stage embryos (Ilic et al., 2009), that is, at an earlier stage than the typical blastocyst. Do blastomere-derived hESCs represent an earlier developmental stage than ICM-derived hESCs, and if so, would equivalent monkey blastomere-derived ESCs contribute to chimeras? Evidence exists that hESCs can acquire a naive state; hESCs derived from ICM under low-oxygen conditions (Lengner et al., 2010) exhibit a more mESC-like phenotype, suggesting that it might be worth exploring whether blastomere-derived monkey ESCs or monkey ESCs derived from early blastocysts under low oxygen acquire a more naive pluripotent state and become chimera competent.

In summary, although other explanations are possible, the inability of monkey ESCs to contribute to chimeric offspring is consistent with other evidence that primate (monkey and human) ESCs display a developmentally advanced epiblast stem cell state, rather than naive

pluripotency. Whether this reflects the developmental status of the ICM at the time of ESC derivation or differential signaling requirements for the maintenance of pluripotency (Roode et al., 2011) should be further explored. In the meantime, extrapolation from mouse pluripotent stem cell data to inform primate stem cell biology is likely to be inadequate. For this reason, it is important to encourage human stem cell biologists to avoid false expectations from other species such as the mouse. However, if primate pluripotent stem cells can be returned to the naive state of pluripotency, their unequivocal value may be further enhanced, and mouse models may become more informative for human medicine.

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Understanding Metastasis in Pancreatic Cancer: A Call for New Clinical Approaches

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Although metastasis is a major cause of morbidity and mortality in patients with pancreatic cancer, the requisite events are currently unknown. In this issue of *Cell*, Haeno et al. and Rhim et al. propose that metastasis occurs much earlier than previously anticipated, with clear implications for improving patient care.

Pancreatic cancer is the most lethal common malignancy, despite standardization of surgical techniques and advances in systemic treatments. Most pancreatic cancer patients present with inoperable disease and rapidly succumb from a devastating illness characterized by tumor spread and vital organ dysfunction, intractable pain, galloping cachexia, and coagulopathy. Surgical resection offers the only hope for cure in the ~20% of patients who qualify, yet few survive longer than 5 or 10 years, and the distinguishing features of this subgroup of patients are unknown. Systemic chemotherapy provides temporary benefits in advanced disease, whereas it prolongs survival measurably in the adjuvant setting presumably by targeting microscopic foci of local and distant disease. Recent intriguing genomic analyses of pancreatic tumors proposed that the initial primary tumor proliferates for several years before producing metastatic clones (Yachida et al., 2010); however, patients with very small or clinically undetectable primary tumors still have a high risk of developing metastases. Therefore, understanding the mechanistic details and temporal pattern of pancreatic cancer metastasis is critical for designing effective interventions, as explored in this issue of *Cell* in two articles by Rhim et al. (2012) and Haeno et al. (2012).

A traditional view of cancer metastasis seeks to identify the ''seed and soil'' factors that may promote this process. Along these lines, it is pertinent to consider the genetic rap sheet of the most common form of pancreatic cancer (pancreatic ductal adenocarcinoma or PDAC), as the four hallmark mutations of PDAC (*KRAS* [>90%], $p16^{INK4A}$ [>90%], *TP53* [~70%], and *SMAD4* [55%]) have all previously been implicated in the metastatic process in human samples and genetically engineered mouse models. Indeed, oncogenic KRAS is known to